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Sexual dimorphism in Tripedaliidae (Conant 1897) (Cnidaria, Cubozoa, Carybdeida)

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Abstract

The family Tripedaliidae was re-defined and expanded based on a molecular phylogenetic hypothesis by Bentlage *et al.* (2010, *Proceedings of the Royal Society Biological Science,* 277: 497). Additionally, Bentlage *et al.* (2010) proposed that all members of the family Tripedaliidae present dimorphism in gonads and have structures that function as seminal vesicles (at least in males). Until now, no information on *Tripedalia binata* concerning gonad morphology, sexual dimorphism, spermatophore formation or structures that serve as seminal vesicles or spermathecae were published. We studied mature medusae of both sexes of *Tripedalia cystophora, Tripedalia binata* and *Copula sivickisi* in order to compare these structures in their stomach regions. We found sexual dimorphism and spermatophore formation in seminal vesicle-like structures in all three species. In particular, we show that along with the females of *Copula sivickisi,* the females of *Tripedalia cystophora* and *Tripedalia binata* also possess structures that store spermatophores and serve as spermathecae.

The results are in agreement with the morphological synapomorphies for Tripedaliidae outlined in Bentlage *et al.* (2010), but suggest an adjustment of the diagnosis of Tripedaliidae (underlined): All carybdeids that display sexual dimorphism of the gonads, produce spermatophores and in which males and females possess subgastral sacs, pockets or purses which function as seminal vesicles or spermathecae.

Key words: *Copula sivickisi*, cubomedusae, gonads, seminal vesicle, spermathecae, spermatophores, *Tripedalia binata*, *Tripedalia cystophora*

Introduction

Until the beginning of the 1970s, box jellyfish were classified as the order Cubomedusae in the class Scyphozoa (Haeckel 1880; Mayer 1910; Stiasny 1919; Thiel 1936; Kramp 1961). During the 1970's cubozoans were studied with a focus on the development, life cycle and morphology (Werner *et al.* 1971; Werner 1973a), culminating with the proposal of raising the order Cubomedusae to the class level among the cnidarians (Werner 1973b; 1975). The new class Cubozoa was established based mostly on the already well-established morphological distinctions—the cuboid shape of the umbrella, presence of pedalia and velarium, and the development of lens eyes in the rhopalia (e.g. Agassiz 1862; Haeckel 1880; Mayer 1910)—from the other groups (Hydrozoa and Scyphozoa) but additionally including life cycle observations from the species *Tripedalia cystophora* Conant, 1897 (Werner *et al.* 1971). Besides the presence of a polyp stage which undergoes complete metamorphosis instead of strobilation that is characteristic of Scyphozoa, the medusae of *Tripedalia cystophora* also display a mating behaviour, which at that time was considered unique among cnidarians.

Cubozoans are the earliest lineage among metazoans to present any kind of copulatory behaviour including internal fertilization. Among cubozoans only members of the family Tripedaliidae Conant, 1897 present sexual dimorphism in the areas where gametes are formed, produce spermatophores and (at least in males) possess subgastral sacs that serve as seminal vesicles (see Hartwick 1991; Bentlage *et al*. 2010).

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Mature females of all three species have the tips of the velarial canals filled with a stark white substance, these prominent spots in *Copula sivickisi* were called "velar spots" and suggested to mark females as mature or immature (Hartwick, 1991; Lewis & Long, 2005). The sperm release and spermatophore production are quite similar in *Copula sivickisi* and *Tripedalia cystophora* even though *Copula sivickisi* produces up to eight spermatophores due to eight subgastric sacs flanking the bases of the gastric phacellae (Hartwick 1991) while *Tripedalia cystophora* produces only four spermatophores in its four stomach purses located near the stomach entrance (Werner 1976). Whether subgastric sacs and stomach purses are homologous structures cannot be stated here with certainty – further studies on morphological development supported by histological sections are planned for the future. Male medusae of *Tripedalia cystophora* stop feeding during spermatophore production (Werner 1976) which might avoid the sperm or the spermatophores being flushed out as the stomach purses are located near the stomach entrance. In contrast, the male medusae of *Copula sivickisi* still feed during spermatophore production which might be possible because the sperm is collected by the gastric filaments into the subgastric sacs located far away from the mouth opening.

We corroborated the possession of subgastric sacs in *Copula sivickisi* and function analogous to the seminal vesicle in males and spermathecae in females, i.e. to form and/or accommodate spermatophores (Hartwick 1991). Werner (1976) described pit-like structures in the males of *Tripedalia cystophora*, which also form and accommodate spermatophores, but he did not mention the same structure for the females. Hartwick (1991, p. 176) denied those structures in females of *T. cystophora* by stating "*Copula sivickisi* is distinct from *Tripedalia* in that ... similar structures, with similar contents (effectively, spermathecae) ... are found in the female as well". However, the females of *Tripedalia cystophora* have four pit-like stomach purses similar to the ones of the males, which might accommodate spermatophores, also shown in *Tripedalia binata*. Further, we show that the females possess additional pockets flanking mesenteries, which we hypothesize serve as spermathecae when the spermatophore membranes are dissolved as observed in females of *Copula sivickisi*.

Morphological examination of the medusae of *Tripedalia binata* shows that both male and female medusae possess stomach purses. In male medusae the purses were occupied by spermatophores, consistent with this species also producing spermatophores and having a mating behaviour similar to the other two species described by Werner (1973a, 1976), Hartwick (1991), Lewis & Long (2005) and Lewis *et al.* (2008).

Our results support the proposal by Bentlage *et al.* (2010), but suggest an adjustment of their amended diagnosis of Tripedaliidae (underlined): All carybdeids that display sexual dimorphism of the gonads, produce spermatophores and in which males <u>and females</u> possess subgastral sacs, <u>pockets or purses</u> which <u>function as seminal vesicles or spermathecae</u>.

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